

FORM-PTO-1390
(Rev. 9-2001)

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER

**TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371**

003300-891

U.S. APPLICATION NO. (if known, see 37 C.F.R. 1.5)

unassigned

10/030429

INTERNATIONAL APPLICATION NO.
PCT/SE00/01491INTERNATIONAL FILING DATE
13 July 2000PRIORITY DATE CLAIMED
13 July 1999, 03 December 1999

TITLE OF INVENTION

USE OF INTERLEUKIN-6 IN TREATMENT OF OBESITY AND/OR OBESITY ASSOCIATED DISORDERS

APPLICANT(S) FOR DO/EO/US

JOHN-OLOV JANSSON and VILLE WALLENIS

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

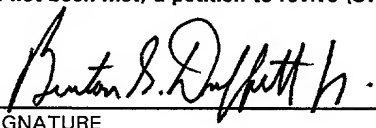
1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. ☐ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
 - a. ☒ is attached hereto (required only if not communicated by the International Bureau).
 - b. ☒ has been communicated by the International Bureau.
 - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2))
 - a. ☐ is attached hereto.
 - b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
 - a. ☐ are attached hereto (required only if not communicated by the International Bureau).
 - b. ☐ have been communicated by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). (Signed Declaration will follow).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 20 below concern document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A **FIRST** preliminary amendment.
14. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter.
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
18. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. ☒ Other items or information: Certified copies of Swedish Application No. 9902680-9, filed 13 July 1999, and Swedish Application No. 9904424-0, filed 03 December 1999, were submitted during the international phase of examination. Thus the claims for priority have been perfected.



21839

U.S. APPLICATION NO. (If known, see 37 CFR 1.51) unassigned 10/030429		INTERNATIONAL APPLICATION NO. PCT/SE00/01491		ATTORNEY'S DOCKET NUMBER 003300-891																	
21. <input type="checkbox"/> The following fees are submitted:				CALCULATIONS	PTO USE ONLY																
Basic National Fee (37 CFR 1.492(a)(1)-(5)): Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to U.S. PATENT AND TRADEMARK OFFICE and International Search Report not prepared by the EPO or JPO \$1,040.00 (960) • International preliminary examination fee (37 CFR 1.482) not paid to U.S. PATENT AND TRADEMARK OFFICE but International Search Report prepared by the EPO or JPO \$890.00 (970) • International preliminary examination fee (37 CFR 1.482) not paid to U.S. PATENT AND TRADEMARK OFFICE but international search fee (37 CFR 1.445(a)(2)) paid to U.S. PATENT AND TRADEMARK OFFICE \$740.00 (958) • International preliminary examination fee (37 CFR 1.482) paid to U.S. PATENT AND TRADEMARK OFFICE but all claims did not satisfy provisions of PCT Article 33(1)-(4) \$710.00 (956) • International preliminary examination fee (37 CFR 1.482) paid to U.S. PATENT AND TRADEMARK OFFICE and all claims satisfied provisions of PCT Article 33(1)-(4) \$100.00 (962)																					
ENTER APPROPRIATE BASIC FEE AMOUNT =																					
Surcharge of \$130.00 (154) for furnishing the oath or declaration later than 20 <input type="checkbox"/> 30 <input type="checkbox"/> months from the earliest claimed priority date (37 CFR 1.492(e)).				\$ 1,040.00																	
<table border="1" style="width:100%; border-collapse: collapse;"> <thead> <tr> <th style="width:20%;">Claims</th> <th style="width:20%;">Number Filed</th> <th style="width:20%;">Number Extra</th> <th style="width:20%;">Rate</th> </tr> </thead> <tbody> <tr> <td>Total Claims</td> <td>22 -20 =</td> <td>2</td> <td>X\$18.00 (966)</td> </tr> <tr> <td>Independent Claims</td> <td>1 -3 =</td> <td>0</td> <td>X\$84.00 (964)</td> </tr> <tr> <td colspan="3">Multiple dependent claim(s) (if applicable)</td> <td>+ \$280.00 (968)</td> </tr> </tbody> </table>				Claims	Number Filed	Number Extra	Rate	Total Claims	22 -20 =	2	X\$18.00 (966)	Independent Claims	1 -3 =	0	X\$84.00 (964)	Multiple dependent claim(s) (if applicable)			+ \$280.00 (968)	\$ --	
Claims	Number Filed	Number Extra	Rate																		
Total Claims	22 -20 =	2	X\$18.00 (966)																		
Independent Claims	1 -3 =	0	X\$84.00 (964)																		
Multiple dependent claim(s) (if applicable)			+ \$280.00 (968)																		
TOTAL OF ABOVE CALCULATIONS =				\$ 1,076.00																	
Reduction for ½ for filing by small entity, if applicable (see below). +				\$ 538.00	-																
SUBTOTAL =				\$ 538.00																	
Processing fee of \$130.00 (156) for furnishing the English translation later than 20 <input type="checkbox"/> 30 <input type="checkbox"/> months from the earliest claimed priority date (37 CFR 1.492(f)). +				\$ --																	
TOTAL NATIONAL FEE =				\$ 538.00																	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 (581) per property +				\$ --																	
TOTAL FEES ENCLOSED =				\$ 538.00																	
				Amount to be refunded: \$																	
				charged: \$																	
<p>a. <input checked="" type="checkbox"/> Small entity status is hereby claimed.</p> <p>b. <input checked="" type="checkbox"/> A check in the amount of \$ <u>538.00</u> to cover the above fees is enclosed.</p> <p>c. <input type="checkbox"/> Please charge my Deposit Account No. <u>02-4800</u> in the amount of \$ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.</p> <p>d. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. <u>02-4800</u>. A duplicate copy of this sheet is enclosed.</p> <p>NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.</p> <p>SEND ALL CORRESPONDENCE TO:</p> <div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>Benton S. Duffett, Jr. BURNS, DOANE, SWECKER & MATHIS, L.L.P. P.O. Box 1404 Alexandria, Virginia 22313-1404 (703) 836-6620</p> </div> <div style="width: 45%; text-align: right;"> <p> SIGNATURE Benton S. Duffett, Jr. NAME <u>22,030</u> REGISTRATION NUMBER <u>January 10, 2002</u> DATE</p> </div> </div>																					

Patent
Attorney's Docket No. 003300-891

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of)
JOHN-OLOV JANSSON et al.) **BOX PCT**
Application No.: (unassigned)) Attention: DO/EO/US
Filed: January 10, 2002) Group Art Unit: (unassigned)
For: USE OF INTERLEUKIN-6 IN) Examiner: (unassigned)
TREATMENT OF OBESITY)
AND/OR OBESITY ASSOCIATED)
DISORDERS)

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

This is a national phase filing of International Application No. PCT/SE00/01491
filed July 13, 2000.

Please amend the above-identified Application as indicated.

IN THE CLAIMS:

Please cancel Claims 1 to 16 without prejudice or disclaimer.

Kindly replace Claims 20, 22 to 26, and 28 as follows:

20. (Amended) A method according to claim 17, wherein said obesity and/or
obesity associated disorders is caused by a pathological disturbance of fat metabolism.

22. (Amended) A method according to claim 17, wherein said obesity is observed despite high levels of circulating leptin.

23. (Amended) A method according to claim 17, wherein said obesity is accompanied by leptin insensitivity.

24. (Amended) A method according to claim 17, wherein said condition is a pathological increase of serum triglycerides.

25. (Amended) A method according to claim 17, wherein said medicinal product is suitable for treatment of a cardiovascular disease.

26. (Amended) A method according to claim 17, wherein said medicinal product is suitable for treatment of a condition due to ageing.

28. (Amended) A method according to claim 17, wherein said IL-6 receptor agonist is administered in combination with a factor that will intensify the effect of said IL-6 receptor agonist.

Please add the following new Claims 31 to 38:

31. (New) A method according to claim 18, wherein said obesity and/or obesity associated disorders is caused by a pathological disturbance of fat metabolism.

32. (New) A method according to claim 19, wherein said obesity and/or obesity associated disorders is caused by a pathological disturbance of fat metabolism.

33. (New) A method according to claim 18, wherein said obesity is observed despite high levels of circulating leptin.

34. (New) A method according to claim 18, wherein said obesity is accompanied by leptin insensitivity.

35. (New) A method according to claim 18, wherein said condition is a pathological increase of serum triglycerides.

36. (New) A method according to claim 18, wherein said medicinal product is suitable for treatment of a cardiovascular disease.

37. (New) A method according to claim 18, wherein said medicinal product is suitable for treatment of a condition due to ageing.

38. (New) A method according to claim 18, wherein said IL-6 receptor agonist is administered in combination with a factor that will intensify the effect of said IL-6 receptor agonist.

003300-891

REMARKS

The present amendment cancels the "use" claims that commonly are not submitted in the United States and modifies the claim format only of Claims 20, 22 to 26, and 28 so as to eliminate the use of multiple dependency.

An Information Disclosure Statement is being filed concurrently herewith.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

By: Benton S. Duffett Jr.
Benton S. Duffett, Jr.
Registration No. 22,030

P.O. Box 1404
Alexandria, Virginia 22313-1404
(703) 836-6620

Filed: January 10, 2002

003300-891

Attachment to Preliminary Amendment dated January 10, 2002

Marked-up Claims 20, 22 to 26, and 28

20. (Amended) A method according to [any one of the claims 17-19] claim 17, wherein said obesity and/or obesity associated disorders is caused by a pathological disturbance of fat metabolism.

22. (Amended) A method according to [according to any one of the claims 17-21] claim 17, wherein said obesity is observed despite high levels of circulating leptin.

23. (Amended) A method according to [according to any one of the claims 17-22] claim 17, wherein said obesity is accompanied by leptin insensitivity.

24. (Amended) A method according to [any one of the claims 17-19] claim 17, wherein said condition is a pathological increase of serum triglycerides.

25. (Amended) A method according to [any one of the claims 17-24] claim 17, wherein said medicinal product is suitable for treatment of a cardiovascular disease.

26. (Amended) A method according to [any one of the claims 17-25] claim 17, wherein said medicinal product is suitable for treatment of a condition due to ageing.

003300-891

Attachment to Preliminary Amendment dated January 10, 2002

Marked-up Claims 20, 22 to 26, and 28

28. (Amended) A method according to [any one of the claims 17-27] claim 17, wherein said IL-6 receptor agonist is administered in combination with a factor that will intensify the effect of said IL-6 receptor agonist.

003300-891

USE OF INTERLEUKIN-6 IN TREATMENT OF OBESITY AND/OR
OBESITY ASSOCIATED DISORDERS

Technical field of the invention

The present invention relates to a new medicinal product and a new method for treatment of pathological disturbances of regulation of body fat tissue mass and/or
5 obesity associated disorders.

Background art

Understanding obesity

Obesity is a large problem in the Western world
10 since both severe and moderate obesity is associated with increased health risks. Obesity is associated with diseases such as diabetes, hypertension and heart disease, whose incidence increases with body-mass index (BMI, body mass in kg/square of height in meters). A study based on
15 information on 18-year-old Swedish military conscripts show a 1.4-fold increase in prevalence of overweight (BMI >25) and a 1.7-fold increase in obesity (BMI >30) from the year 1971 to 1993 (Rasmussen F, Johansson M and Hansen HO, 1999).

20 Generally, obesity is due to energy intake that exceeds energy expenditure. This can be caused by overeating, i.e. higher food intake than necessary for maintenance of body mass. In addition, low mobility and low metabolic rate may predispose for obesity (see Flier,
25 J. S. and Foster D. W. (1998) Eating disorders: obesity, anorexia nervosa, and bulimia nervosa. In: Williams Textbook of Endocrinology, 9th Ed, Saunders Co.).

However, the general opinion that obesity is largely the result of a lack of willpower is unsatisfactory. Intense research efforts are therefore made to reveal the
30 genetic and environmental factors of importance for development of obesity (Friedman JM and Halaas JL, 1998).

Obesity in humans and mice

Animal models can be used for investigation of which genes that are causing development of obesity. Of particular importance is the information that can be gained from mouse strains that develop obesity because of gene knockouts. These mouse strains can provide evidence that a certain gene product is of crucial importance for regulation of body fat. This in turn may facilitate the development of new treatment paradigms. There are indications that there are gender differences regarding the genetic ethiology of obesity (see e.g. Costet, P. et al. (1998) Peroxisome Proliferator-activated receptor α -isoform deficiency leads to progressive dyslipidemia with sexually dimorphic obesity and steatosis. J. Biochem. Chem. 273,29577-29585).

Obesity and blood fats in relation to cardiovascular disease

It is recognized that obesity, especially visceral obesity, and deranged lipid-lipoprotein profile, including hypertriglyceridemia and hypercholesteolemia are associated with larger risk of cardiovascular disease (Lamarche B, et al. (1998), Visceral obesity and the risk of ischemic heart disease: insights from the Quebec cardiovascular study. Growth hormone and IGF research 8, (suppl. B) 1-8.). So far, a lot of the research on the ethiology of this syndrome has dealt with neuroendocrine, i.e. hypothalamohypophyseal, and endocrine disturbances, focusing on the effects of the hypothalamus-pituitary-adrenal (HPA) axis regulating glucocorticoid, sex steroids and growth hormone (see e.g. Björntorp, P. (1996) The regulation of adipose tissue distribution in humans, Int. J. Obesity 20, 291-301.)

Leptin and obesity

Following the cloning of leptin 6 years ago (see Zhang et al. (1994), Positional cloning of the mouse ob

(obesity) gene and its human homologue. Nature 372, 425-432), there were great hopes that this would mean new possibilities to treat obesity and overeating. However, later it was found that obesity in humans very seldom is due to leptin deficiency, but rather is associated with increased leptin levels. Moreover, it has been shown that both mice and humans often are resistant to the anti-obesity effect of leptin (see e.g. Flier, J. S. (1998), What's in a name? In search of leptin's physiological role, J Clin. Endocr. Metab 83, 1407-1413, and references therein).

The 16 kDa protein leptin is almost only produced in white adipocytes from which leptin is then released to circulation. Leptin production by fat and plasma leptin levels is highly correlated with adipose tissue mass (Flier JS, 1997). Leptin acts through specific receptors in the hypothalamus to create a feedback loop for body weight regulation. Therefore, the pathophysiology of obesity was assumed to be partly endocrine. Leptin does not rise significantly after a meal and does not result in the termination of a meal. Instead leptin appears largely to exert long-term effects on food consumption and energy expenditure (Flier JS, 1998; Friedman JM and Halaas JL, 1998).

25

Leptin as a starvation signal

Obese (*ob*) mice which lack leptin show many of the abnormalities seen in starved animals, including hyperphagia, decreased body temperature, decreased energy expenditure, decreased immune function, and infertility. Leptin replacement corrects all of these abnormalities implying that *ob* mice live in a state of "perceived starvation" due to lack of leptin and that the biological response in the presence of food leads to obesity. These observations led to speculation that leptin's main physiological role is to signal nutritional status during

periods of food deprivation (Flier JS, 1998; Friedman JM and Halaas JL, 1998).

The leptin receptors

5 The leptin receptor (Ob-R) is normally expressed at high levels in hypothalamic neurons and in other cell types, including T cells and vascular endothelial cells. *In situ* hybridisation was used to identify the hypothalamic arcuate nucleus, and also dorsomedial hypothalamic
10 nucleus (DMH), paraventricular nucleus (PVN), ventromedial hypothalamic nucleus (VMH) and lateral hypothalamic nucleus (LH) as principal sites of Ob-R expression in the central nervous system. Each of these nuclei, such as the arcuate nucleus, express one or more neuropeptides and
15 neurotransmitters such as neuropeptide Y (NPY) and melanocyte-stimulating hormone alpha (-MSH), that regulate food intake and/or body weight, probably by actions downstream of leptin (Friedman JM and Halaas JL, 1998; Flier JS and Maratos-Flier E, 1998).

20

Leptin and human obesity

 The role of leptin in the pathogenesis of obesity may be inferred by measurement of plasma leptin. An increase in plasma leptin suggests that obesity is the result of resistance to leptin. A low or normal plasma concentration of leptin suggests that obesity is due to decreased production of leptin. This interpretation is
25 similar to that used in studies of insulin and the pathogenesis of type I and type II diabetes. As is the case with insulin and its receptor in diabetes, mutations of
30 leptin and its receptor are rare in human obesity, but most obese individuals still have higher levels of leptin than do non-obese individuals, an indication of leptin resistance that might be receptor-independent (Flier JS,
35 1997).

 Many genes involved in development of obesity have recently been found and most of them seem to act down-

stream of leptin at the hypothalamic level. Other genes that are involved in development of obesity encode neuro-peptides, e.g. leukocyte adhesion receptors, which are important cell-cell adhesion molecules in the inflammatory and immune systems (Dong ZM et al., 1997), and neurocytokines like ciliary neurotrophic factor, whose receptor subunits share sequence similarity with the leptin receptor (Gloaguen I et al., 1997). The identification of anti-obesity mechanisms that act independently or together with the leptin system may help to develop strategies for the treatment of obesity associated with leptin resistance.

Leptin has immuno-regulatory activity

Exogenous leptin up-regulates both phagocytosis and the macrophage production of proinflammatory cytokines such as tumor necrosis factor (TNF-) and interleukin-6 (Loffreda S et al., 1998). It has been suggested that the up-regulation of inflammatory immune responses by leptin may contribute to several of the major complications of obesity such as increased incidence of infection, diabetes and cardiovascular disease (Loffreda S et al., 1998; McCarty MF, 1999). This hypothesis is attractive since it would implicate a common pathogenic mechanism (lack of leptin action) for both obesity and some of its major complications. However, an alternative possibility is that regulatory mechanisms usually connected to e.g. immune functions also are of importance for the regulation of body fat.

Interleukin-6

The cytokines act as hormonal regulators of the immune system and in the body's reactions during trauma and inflammation. The cytokine interleukin-6 (IL-6) is known to be important in the development of B-lymphocytes and in the change of plasma protein production of the liver during trauma and inflammation, the so-called acute phase

response. In line with this, IL-6 levels are markedly increased during acute phase response. It has been shown that IL-6-type cytokine receptors share functional specificity with the long form of the leptin receptor (Baumann H et al., 1996). The role of the cytokines including IL-6 in healthy animals and humans is not well known and they are suggested to have little effect, partly because circulating levels often are low in the absence of illness (Hirano T, 1998).

10

Structures of interleukin-6 and its receptor

Interleukin-6 (IL-6) exerts its biological effects through the ligand-specific IL-6 receptor, which belongs to the cytokine receptor superfamily. The multisubunit IL-6 receptor complex consists of the IL-6R α subunit which binds to IL-6 and the membrane associated glycoprotein gp130 which is a signal transducer. Unlike most other cytokine receptors, the IL-6R α subunit can be activated by ligand binding in both its membrane bound and its soluble form. IL-6 induces heterodimerization between IL-6R α and gp130, which in turn leads to homodimerization of gp130 to a second gp130 molecule (see e.g. Hirano, T. (1998), Interleukin 6 and its receptor: ten years later. Int. Rev. Immunol. 16, 249-284). Actually, IL-6/IL-6R α complexes can be potent activators of gp130, including in cells that lack membrane bound IL-6R α . Since gp130 can be activated by several other ligand-receptor complexes, these effects may not reflect the physiological role of IL-6 (see e.g. Schirmacher, P., et al. (1998), Hepatocellular hyperplasia, plasmacytoma formation, and extramedullary hematopoiesis in interleukin (IL)-6/soluble IL-6 receptor double-transgenic mice. Am. J. Pathol. 153, 639-648). On the other hand, the fact that several different types of cytokine receptors can activate gp130 opens the possibility that different cytokines may potentiate each others actions thereby exerting synergistic effects. One example of receptors belonging to the IL-6R α family is

the leptin receptor (Tartaglia, L. A. et al., (1995), Identification and expression cloning of a leptin receptor, OB-R. Cell 83, 1263-1271) but the leptin receptor is not acting via gp130 (see e.g. Baumann, H., (1996), The full-length leptin receptor has signaling capabilities of interleukin 6-type receptors. Proc Natl. Acad. Sci. USA 93, 8374-8378).

Most patents issued regarding IL-6 have described methods to get beneficial effects of suppression of IL-6 action. One exception is a recent patent claiming that IL-6 can suppress demyelination, e.g. during multiple sclerosis (see US Pat. No. 5,863,529) Methods have been developed for production of human IL-6 in large quantities (see e.g. US Pat. No. 5,641,868).

Interleukin-6 agonists

Several IL-6 have been described in previous patent applications. For instance, possible superagonists made from wild type human IL-6 with various amino acid substitutions have been described (see e.g. US Pat. No. 5,914,106, US Pat. No. 5,506,107, and US No. 5,891,998).

Interleukin-6 and obesity

It has recently been discovered that knockout of the IL-6 gene in mice surprisingly induces "middle age onset" obesity (Wallenius V and Jansson JO, unpublished results). There is little data in the literature indicating that IL-6 has any effect on metabolic parameters in the absence of acute phase reaction and inflammation. However, there are recent reports indicating that IL-6 is released from normal adipose tissue in humans. In addition, the IL-6 levels in blood are proportional to body fat mass (Mohamed-Ali V et al., (1997), Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-alpha, in vivo. *J Clin Endocrinol Metab* 82, 4196-200). If IL-6 prevents obesity, this finding suggest that obese individual could be IL-6 resistant,

and therefore benefit from treatment with a factor that enhances the effect of IL-6 in addition to IL-6 itself. In addition, it is well known that IL-6 is released from immune cells including macrophages, as well as endothelial cells and various other cell types (Hirano T, 1998). Moreover, both IL-6 and IL-6 receptors have been found in hypothalamic nuclei known to be important in the regulation of food intake and body weight (Schöbitz B et al., 1993, see Fig. 1). These observations have drawn our attention to IL-6's potential role in the regulation of body weight.

IL-6 and acute phase reaction (APR)

IL-6 plays a role for different parts of the immune response (see e.g. Hirano, T. (1998), supra). It is well known that production of IL-6 as well as the circulating levels of this cytokine is enhanced during so-called acute phase reaction (APR). Moreover, IL-6 is considered a key mediator of APR, especially after infection with gram positive bacteria (see e.g. Kopf, M., et al. (1994), Impaired immune and acute-phase responses in interleukin-6-deficient mice. Nature 368, 339-342). The APR is characterized by changes in the composition of the proteins released into plasma from the liver. APR is seen in pathological conditions with an inflammatory component such as trauma, infections, autoimmune disease, and tumors. These conditions are also associated with catabolism, i.e. decreased growth and increased degradation of tissues belonging to the fat free mass in the body.

30

IL-6 and ageing

Aging is associated with several somatic changes including increased body fat mass in general and visceral fat mass in particular (see e.g. Rudman, D., et al., (1990), Effects of human growth hormone in men over 60 years old. N. Engl. J. Med. 323, 1-6; Flier, J. S. and Foster D. W. (1998) supra). The proportion of the popula-

35

tion that have disturbances of blood fats such as pathologically elevated serum triglycerides also increase with age and is higher in middle aged than in young adult persons (Brown, M. S., and Goldstein, J. L. (1983) Disorders of lipid metabolism, Harrison's principle of internal medicine, 10th Ed, 547-559. It has been suggested that several age-associated diseases are caused by enhanced IL-6 (see e.g. Ershler, W. B., et al., (1994), The role of interleukin-6 in certain age-related diseases. Drugs Aging 5, 358-365). In humans there is an epidemiological connection between high IL-6 levels in peripheral blood mononuclear cells (PBMC) (see e.g. O'Mahony, L., et al., (1998), Quantitative intracellular cytokine measurement: age-related changes in proinflammatory cytokine production. Clin. Exp. Immunol. 113, 213-219) as well as in serum (see e.g. Mysliwska, J., et al., (1998), Increase of interleukin 6 and decrease of interleukin 2 production during the aging process are influenced by the health status. Mech. Aging Dev. 100, 313-328).

Effects of low, normal levels of IL-6 in mice of different age

There is much information about the effects of high levels of IL-6, e.g. in connection with inflammation (see e.g. Kopf, M., et al, supra). However, little is known about the importance of the low, basal levels of IL-6 in animals and humans without inflammation. One reason could be that it has been difficult to measure the low IL-6 levels in healthy mice with the assays available today. However, it can not be excluded that there still is a biologically significant effect of IL-6 in these animals. Moreover, IL-6 that is produced locally in tissues may exert autocrine or paracrine effects on cells in the same tissue, without being transported to other organs via blood circulation.

There have been few reports of differences between mice with complete IL-6 deficiency due to targeted dis-

ruption of the IL-6 gene, and normal wild type mice in the absence of provocations (see e.g. Hirano, T. (1998), supra). It is known that these mice develop normally to adulthood and they are fertile (see e.g. Kopf, M., et al., supra, and Poli, V., et al., (1994). Interleukin-6 deficient mice have been reported to be protected from bone loss caused by estrogen depletion. EMBO J. 13, 1189-1196). It has also been reported that IL-6 mice might have a defective fever response (see e.g. Hirano, T. (1998), supra). However, very little has been published about the effects of IL-6 deficiency in mice that are older than a couple of months. This could be due to the fact that it is expensive and laborious to keep mice for longer time. Since the normal life span of a mouse is about two years, there are few publications about a large part of the adult life of mice.

Regulation of IL-6 production and release

As mentioned above, IL-6 is released during acute phase reaction. Therefore, it is not surprising that IL-6 production is enhanced by gram-positive as well as by gram-negative bacteria. The latter seem to release IL-6 via production of an antigen called lipopolysaccharide (LPS) (see e.g. Kopf, M., et al. (1994), supra). The production of IL-6 is enhanced by tumor necrosis factor- α , TNF- α , a cytokine that is thought to play a role for the induction of type 2 diabetes, an illness associated with visceral obesity and cardiovascular disease. TNF- α production is enhanced from adipocytes that have accumulated fat (see e.g. Hotamisligil G. S. and Spiegelman B. M., (1994), Tumor necrosis factor alpha: a key component of the obesity-diabetes link. Diabetes 43, 1271-1278; Flier, J. S. and Foster D. W. (1998), supra).

Several other hormones have also been shown to enhance IL-6 production. These include parathyroid hormone (PTH), 1,25-dihydroxyvitamin D3, thyroid hormone, platelet-derived growth factor, insulin-like growth factor I,

and IL-1 (see e.g. Swolin, D., et al., (1996), Growth hormone increases interleukin-6 produced by human osteoblast-like cells. J. Clin. Endocrinol. Metab. 81, 4329-4333, and references therein). In addition, it has been shown that nicotine, a well known suppresser of obesity, can enhance IL-6 production and plasma IL-6 levels (see e.g. Song, D-K., et al., (1999), Central injection of nicotine increases hepatic and splenic interelukin-6 (IL-6) mRNA expression in mice: involvement of the peripheral sympathetic nervous system. FASEB J13:1259-1267). It has also been reported that corticosteroids, which are well known inducers of visceral obesity, can suppress IL-6 expression (see e.g. Swolin-Eide, D., et al., (1998), Effects of cortisol on the expression of interleukin-6 and interleukin-1 beta in human osteoblast-like cells. J. Endocrinol. 156, 107-114).

IL-6 and body fat during APR

IL-6 is a major mediator of APR, a condition associated with wasting and decreased appetite. However, it is still by no means certain that IL-6 also causes these anorectic and wasting effects. In fact, there are data indicating that this is not the case, although lipopolysaccharides (LPS) were reported to induce weight loss in mice and that this effect can be significantly prevented by treatment with anti-IL-6 monoclonal antibodies. However, in the same study the anti-IL-6 antibodies did not prevent the hypertriglyceridemia induced by LPS, possibly suggesting that IL-6 is less important for changes in fat metabolism during APR (Strassman, G. et al. (1993), The role of interleukin-6 in lipopolysaccharide-induced weight loss, hyperglycemia and fibrinogenproduction. Cytokine 5, 285-290).

It has been reported that IL-6 treatment can decrease lipoprotein lipase (LPL) activity in adipose tissue of mice and in murine adipocyte cell lines in vitro. This effect has been seen as an indication of a lipolytic

effect of IL-6 during cancer cachexia, a condition associated with APR (see Greenberg, A. S., (1992), Interleukin-6 reduces lipoprotein lipase activity in adipose tissue of mice in vivo and in 3T3-L1 adipocytes: a possible role for interleukin-6 in cancer cachexia, Cancer Res. 52, 4113-4116). On the other hand, there are indications e.g. from studies of gene knockout mice that LPL activity does not affect fat accumulation (Zechner, R (1997), The tissue specific expression of lipoprotein lipase: implications for energy and lipoprotein metabolism, Curr. Opin. Lipidol. 877-88).

IL-6 and body fat during normal conditions

It has been speculated that that IL-6, like leptin, could have an adipostatic activity also in patients without APR. However, this assumption was based only on the finding that subcutaneous fat releases IL-6 in patients without acute phase reaction. Not surprisingly, there was also a correlation between high BMI, presumably reflecting fat mass, and levels of circulating IL-6 (Mohamed-Ali, V., et al. (1997) Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor- α , in vivo, J. Clin. Endocrinol. Metab. 82,4196-4200). However, the finding that IL-6 is released by adipose tissue, does in no way prove that this factor would regulate fat tissue mass. As noted above, it is by no means clear that IL-6 is of importance for lipolysis even during APR. In the absence of APR, the available data has suggested that long term treatment with IL-6 in low, physiological doses is not lipolytic by itself. Although a single injection of IL-6 in a dose of 50 μ g/kg body weight has been shown to enhance release of free fatty acids into blood circulation (Nonogagi K, et al. (1995), Interleukin-6 stimulates hepatic triglyceride secretion in rats, Endocrinology 136, 2143-2149), there is no obvious loss of fat mass in transgenic mice with very high levels of circulating IL-6 (see e.g. Peters, M. (1997), Extramedullary expan-

sion of hematopoietic progenitor cells in interleukin (IL)-6-sIL-6R double transgenic mice. J. Exp. Med. 185,755-766), although such mice display growth impairment (De Benedetti, F. et al. (1997), Interleukin-6 causes growth impairment in transgenic mice through a decrease in insulin-like growth factor-1. J. Clin. Invest. 99, 643-650) as well as muscle atrophy (Tsujinak, T et al. (1996), Interleukin 6 receptor antibody inhibits muscle atrophy and modulates proteolytic systems in interleukin 6 transgenic mice. J. Clin. Invest. 97, 244-249). Moreover, there have been few indications in the literature that long term absence of the low physiological amounts of endogenous IL-6 that are produced in an animal or human without APR, would have consequences for fat metabolism, especially fat mass and blood fat levels. The best way to investigate the consequences of long term absence is probably the study of mice with IL-6 gene knock out. In 1998 one of the worlds leading experts on IL-6 concluded in a review that the results of IL-6 knock out in mice had shown "that IL-6 is critical in only a limited range of biological reactions such as APR, the mucosal IgA response, the fever response, and estrogen deficiency-induced bone loss." (see e.g. Hirano, T. (1998), supra, p 252). No effects of fat mass in IL-6 knock-out mice have been reported. As noted above, IL-6 can suppress LPL (see Greenberg, A. S., (1992), supra), and it has also been suggested that LPL can increase predisposition for obesity and fat accumulation. On the other hand, this theory is challenged by the fact that fat specific deletion of LPL activity does not affect fat mass (Zechner, R (1997), The tissue specific expression of lipoprotein lipase: implications for energy and lipoprotein metabolism, Curr. Opin. Lipidol. 8,77-88). The general opinion by well renowned researchers today is that IL-6 does not affect fat mass essentially, especially not during normal life without APR.

IL-6 and ethanol

Under certain circumstances, alcohol can suppress the concentration of circulating IL-6 (see e.g. Akerman, P. A., et al. (1993), Long-term ethanol consumption alters the hepatic response to the regenerative effects of tumor necrosis factor-alpha. *Hepatology* 17, 1066-1073). It is also well known that ethanol can cause visceral obesity as well as deranged blood fats including enhanced serum triglyceride levels (Brown, M. S., and Goldstein, J. L. (1983), *supra*).

TNF- α and regulation of body fat

As mentioned above, TNF- α is a stimulator of IL-6 production. This effect of TNF- α is exerted via the type 1 (p55) receptor, since it has been shown that IL-6 levels are decreased in mice with TNF receptor 1, but not TNF receptor 2, gene knock out (Yamada, Y., et al. (1998), Analysis of liver regeneration in mice lacking type 1 or type 2 tumour necrosis factor receptor: requirement for type 1 but not type 2 receptor. *Hepatology* 28,959-970). The role of TNF- α for development of obesity is not clear. Mice lacking the TNF- α ligand have not been reported to be obese (Uysal, K. T., et al (1997), Protection from obesity induced insulin resistance in mice lacking TNF- α , *Nature* 389,610-614), and there was no obesity in mice deficient in the both of the two receptors, type 1 (p55) and type 2 (p75), that are thought to mediate the biological effects of TNF- α . Actually, mice deficient in the type 2 (p75) receptor gain less weight when given high fat diet, suggesting that TNF- α might even stimulate obesity via this receptor type (Schreyer, S. A. (1998), Obesity and diabetes in TNF- α receptor deficient mice. *J. Clin. Invest.* 102,402-411). Furthermore, no increase in body weight was found in mice with TNF receptor 1 gene knock out even when they were fed high fat diet (Schreyer, S. A. (1998), *supra*). Obesity in *db/db* (*diabetes/diabetes*) mice with a defective leptin recep-

tor, was not affected by lack of the TNF receptor 1 (Schreyer, S. A. et al (1998), supra) or by lack of the ligand TNF- α which activates both receptor 1 and receptor 2 (Uysal, K. T., et al., (1997), supra). Another finding
5 that argues against beneficial effects of TNF- α in obesity is that TNF- α often enhances insulin resistance, a symptom often associated with obesity (see Flier, J. S. and Foster D. W. (1998), supra).

10 Cytokines and atherosclerosis

Although the interest in the possible associations between cytokines and atherosclerosis has increased during recent years, it has mostly concerned the possible deleterious effects of cytokines and inflammation in development of atherosclerosis. The cytokines have been assumed to stimulate the development of the atherosclerotic
15 plaques by local effects (see e.g. Rus, H. G., et al., (1996) Interleukin-6 and interleukin-8 protein and gene expression in human arterial atherosclerotic wall. Atherosclerosis 127,263-271). In addition, as mentioned
20 above, IL-6 has been reported to increase circulating triglycerides by release of triglycerides from the liver (Nonogagi K, et al. (1995), Interleukin-6 stimulates hepatic triglyceride secretion in rats, Endocrinology 136, 2143-2149).
25

Summary of the invention

The object of the present invention is to provide new medicinal products and methods for treatment of obesity and/or obesity associated disorders.
30

The invention relates to the use of a substance that upon administration to a patient will lead to an increased level of an interleukin-6 (IL-6) receptor agonist for the production of a medicinal product for the treatment of obesity and/or obesity associated disorders.
35

Furthermore, the invention relates to a method for treatment of obesity and/or obesity associated disorders

wherein a pharmaceutically effective amount of a substance that upon administration to a patient will lead to an increased level of an interleukin-6 (IL-6) receptor agonist is administered to said patient.

5 The characterizing features of the invention will be evident from the following description and the appended claims.

Detailed description of the invention

10 In the research work leading to the present invention it was found that endogenous IL-6 can inhibit development of "middle-aged"-onset obesity as well as obesity associated disorders, e g the metabolic syndrome. The metabolic syndrome (also called syndrome X) comprises
15 obesity (in particular abdominal obesity), disturbances of blood fats (e g triglycerides), and diabetes type II.

 The invention thus relates to medicinal products comprising a substance that upon administration to a patient will lead to an increased level of an interleukin-6
20 (IL-6) receptor agonist. Said substance may be an IL-6 receptor agonist. A preferred example of such an agonist is IL-6. It is possible to use a naturally occurring agonist, such as IL-6, as well as a synthetically produced agonist, such as an IL-6 mimetic. Examples of synthetically produced IL-6 receptor agonists are given in US 550
25 61 07 (Cunningham et al), US 589 19 98 (Rocco et al), and US 591 41 06 (Gennaro et al). Said substance may also be a substance that upon administration will lead to the release of an endogenous occurring IL-6 receptor agonist,
30 preferably IL-6, from different cells, such as endothelial cells, or organs, such as the liver.

 The expression "IL-6 receptor agonist" used herein relates to all substances that bind to and activate the same receptor proteins as IL-6.

35 The term "patient" used herein relates to any human or non-human mammal in need of treatment with the medicinal product or method according to the invention.

The term "treatment" used herein relates to both treatment in order to cure or alleviate a disease or a condition, and to treatment in order to prevent the development of a disease or a condition. The treatment may
5 either be performed in an acute or in a chronic way.

As mentioned above, the invention is suitable for treatment of high levels of triglycerides. The expressions "high levels of triglycerides" relates to amounts of this compound that are higher than for a normal,
10 healthy person.

The medicinal product and the method according to the invention are suitable for treatment of different pathological disturbances of regulation of body fat tissues, leading to obesity and/or obesity associated disorders. One example is visceral or general obesity that is
15 due to genetic predisposition, a condition sometimes described as the thrifty genotype. Another example is diet-induced obesity, a condition that often is resistant to leptin treatment.

The medicinal product and the method according to the invention are e.g. suitable for treatment of cardiovascular disease, since obesity and obesity associated disorders are associated with an increased risk of cardiovascular disease.
20

The medicinal product and the method according to the invention are also suitable for treatment of persons that have been exposed to high doses of glucocorticoid hormone, e.g. due to tumours producing such hormones, due to treatment with glucocorticoids against certain diseases, or due to abuse of glucocorticoids. It is known
25 that high levels of glucocorticoids cause visceral obesity and disturbed blood fats. It has been shown that glucocorticoids under certain circumstances can decrease IL-6 production.
30

Other patients which may be treated with the medicinal product or the method according to the invention are persons with obesity, obesity associated disorders,
35

and/or low endogenous production of IL-6 during normal state, i.e., in the absence of APR. Also persons with obesity and/or obesity associated disorders in combination with insensitivity to IL-6 may be treated with the medicinal product and the method according to the invention. The IL-6 insensitivity could e.g. be caused by low levels of the receptor protein IL-6R α on the cell surface or low levels of the glycoprotein gp130 which normally mediates the effects of IL-6. In these persons, the IL-6 produced by the patients themselves may not be sufficient to inhibit development of obesity and/or obesity associated disorders.

Another example of a group of patient which may be treated according to the invention are patients suffering from normal aging. In some cases, the production of IL-6 in important tissues could be insufficient although the circulating levels often are increased. A possible IL-6 insufficiency in aging may also be due in part to insensitivity to IL-6.

It is also possible to treat patients with obesity and/or obesity associated disorders in combination with low concentrations of growth hormone (GH) receptors or defective GH receptors. It is known that GH has lipolytic effects.

It is also possible to treat obese patients with low concentrations of leptin or leptin receptors, or patients with defective leptin receptors. More often, it would be beneficial to treat patients with obesity and/or obesity associated disorders in combination with leptin resistance due to unknown reasons.

Also patients abusing alcohol may suffer from conditions treatable according to the present invention. It has been shown that alcohol may decrease IL-6 levels (Akerman, P. A., et al. (1993), supra) and that patients abusing alcohol often display increase visceral obesity and enhanced serum triglyceride levels in man (Flier, J. S. and Foster D. W. (1998), supra).

It may be advantageous to combine the substance that upon administration to a patient will lead to an increased level of an interleukin-6 (IL-6) receptor agonist used according to the invention with a factor that will intensify the effect of said interleukin-6 (IL-6) receptor agonist, and the medicinal product according to the invention may thus also comprise such a factor. An example of such a factor is a soluble IL-6 binding protein. However, a problem may be that IL-6 in combination with soluble IL-6R α may exert unspecific effects, including even on cells that do not have membrane bound IL-6R α (see e.g. Peters, M. (1997), supra).

The medicinal product according to the invention may also comprise other substances, such as an inert vehicle, or pharmaceutical acceptable adjuvants, carriers, preservatives etc., which are well known to persons skilled in the art.

The medicinal product according to the invention may be formulated for enteral (e.g. oral or per oral) or parenteral administration.

The invention also relates to use of a substance that upon administration to a patient will lead to an increased level of an interleukin-6 (IL-6) receptor agonist for a medicinal product for treatment of the above specified conditions.

Furthermore, the invention relates to a method for treatment of pathological disturbances of fat metabolism wherein a pharmaceutically effective amount of a substance that upon administration to a patient will lead to an increased level of an interleukin-6 (IL-6) receptor agonist is administered to said patient. Preferably, said substance is administered together with a factor that will intensify the effect of said interleukin-6 (IL-6) receptor agonist.

Since these effects of IL-6 on fat metabolism were first seen in the work leading to the present invention after removal of endogenous IL-6, it seems appropriate to

use IL-6 according to the invention in doses that previously have been used to substitute for IL-6 deficiency. Such a dose would be about 1 mg/kg body weight given as a subcutaneous injection to mice (see e.g. e.g. Cressman, D. E., et al., (1996), Liver failure and defective hepatocyte regeneration in interleukin-6-deficient mice. Science 274, 1379-1383). However, the dose of IL-6 in humans could be quite different. The dose may be higher in older individuals, since it has been shown that IL-6 levels increase with age. The dose may be lower than those doses that would result in IL-6 levels found during APR, to avoid side effects similar to the symptoms of APR.

The invention will now be further explained in the following examples. These examples are only intended to illustrate the invention and should in no way be considered to limit the scope of the invention.

Brief description of the drawings

In the examples below reference is made to the accompanying drawing on which:

Fig 1 A shows the effect of interleukin-6 gene knock out in male mice on mean body weight at different ages. Fig 1 B shows the physical appearance of IL-6 knock out male mice at 9-10 months of age. The photo shows representative body shapes of IL-6 -/- and IL-6 +/+ male mice. The computerized tomography (CT) shows transverse sections of the abdomen of representative IL-6 -/- and IL-6 +/+ male mice (C).

Fig 2 A, B and C illustrates the effects of interleukin-6 gene knock out on mean body weight at different ages in female mice (Fig 2 A) and the effect of interleukin-6 gene knock out on mean body mass index (Fig 2 B) ($\text{BMI, body weight}/(\text{crown-rump length})^2$) and mean visceral transversal width (mm) (Fig 2 C) were also investigated in 9 month-old female mice.

Fig 3 Shows the measured daily food intake during three consecutive days in 11 month-old female IL-6 +/+ and IL-6 -/- mice.

Fig 4 A and B illustrates the effects of interleukin-6 gene knock out in female mice on serum triglyceride levels (Fig 4 A) and serum leptin levels (Fig 4 B).

Fig 5 shows the possible sources of IL-6 that could be of importance for body composition and leptin sensitivity.

Fig 6 shows the effect of vehicle and leptin administration on food intake in 15 month-old wild-type and IL-6 knockout (IL-6^{-/-}) male mice. 8 A shows vehicle treated mice, wild-type $n = 5$, IL-6^{-/-} $n = 4$. 8 B shows leptin at 120 $\mu\text{g}/\text{day}$, $n = 5$ per genotype. 8 C shows leptin at 240 $\mu\text{g}/\text{day}$, wild-type $n = 5$, IL-6^{-/-} $n = 3$. Thick black bars represent leptin treatment period. Vehicle or leptin was injected intraperitoneally twice daily. Values are indicated as mean \pm SEM. # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$ vs. study day 0, paired t test with the Bonferroni correction. * $P < 0.05$, ** $P < 0.01$ vs. wild-type, independent t test.

Fig 7 shows the effect of vehicle and leptin administration on body weight in 15 month-old wild-type and IL-6 knockout (IL-6^{-/-}) male mice. 9 A shows vehicle treated mice, wild-type $n = 5$, IL-6^{-/-} $n = 4$. 9 B shows leptin at 120 $\mu\text{g}/\text{day}$, $n = 5$ per genotype. 9 C shows leptin at 240 $\mu\text{g}/\text{day}$, wild-type $n = 5$, IL-6^{-/-} $n = 3$. Thick black bars represent leptin treatment period. Vehicle or leptin was injected intraperitoneally twice daily. Values are indicated as mean \pm SEM. # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$ vs. study day 0, paired t test with the Bonferroni correction. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. wild-type, independent t test.

Fig 8 shows relative weights of different fat depots (% fat weight/body weight) in IL-6^{+/+} and IL-6^{-/-} mice. Three intra-abdominal fat pads (gonadal, retroperitoneal and mesenteric) and the femoral fat pad

(a subcutaneous fat pad on the outer thigh) were dissected and weighed in 18-month-old male (A) and female (B) IL-6^{+/+} and IL-6^{-/-} mice. There were 4-10 mice in each group. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$, vs. corresponding IL-6^{+/+} mice.

Fig 9 shows comparison of the effect of IL-6 treatment in IL-6^{+/+} and IL-6^{-/-} mice. The mice were treated with gradually increasing doses of IL-6 (40 ng/day, days 0-4; 80 ng/day, days 5-12; 160 ng/day, days 13-18). Changes in body weight (g) during the IL-6 treatment period compared to before start of treatment (A). Figures 11 B and C compare values at day 0 before initiation of IL-6 treatment with day 18 after IL-6 treatment in IL-6^{+/+} and IL-6^{-/-} mice. The total abdominal area was calculated from the CT scans (B). The intraperitoneal area containing fat was measured separately by calculating the darker areas with attenuation similar to subcutaneous fat (C). Both the total intraperitoneal and intraperitoneal fat areas were calculated blindly by two different people, with no connection to the study. There were 5 mice in each group. All animals were 12-month-old at the start of the treatment. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$, vs. corresponding control mice. # $P < 0.05$, vs. the corresponding group before initiation of IL-6 treatment.

25

Examples

The IL-6 knock out mice (i.e. IL-6^{-/-} mice) and the corresponding controls used in these examples were kindly provided by Dr. Manfred Kopf at Basel Institute of Immunology, Basle, Switzerland (see e.g. Kopf, M. (1994), supra). The IL-6^{-/-} mice were back crossed 7-8 times with c57Bl/6 mice to gain a strain of mice genetically consisting of more than 95 % c57Bl/6.

As controls to the IL-6^{-/-} mice, wild type c57Bl/6 mice (i.e. IL-6^{+/+} mice) (Bomholtgård Breeding & Research Centre A/S) were used in examples 1-4. These mice were kept at standardized conditions with standard low

fat chow and water freely available. Food intake was measured keeping two female mice per cage. The amount of chow was recorded once per day. In example 5 age-matched normal C57BL/6 male mice from B&K Universal AB (Sollentuna, Sweden) were used as wild-type controls. All male mice were housed separately (due to aggressiveness) in standard cages under standardised environmental conditions, i.e. 24-26°C, 50-60% relative humidity, artificial lightning at 05:00-19:00 hours, with water and pelleted food (Beekay Feeds, Rat and mouse standard diet, B&K Universal AB, Sollentuna, Sweden) *ad libitum*.

In examples 6 and 7, mice with IL-6 gene knock-out (IL-6^{-/-} mice) were generated as described by Kopf et al (12). To reduce genetic heterogeneity, the IL-6^{-/-} genotype was moved onto C57BL/6 background by eight successive back crosses. The resulting strain of mice consists genetically of more than 99.5% C57BL/6. Normal C57BL/6 mice from B&K Universal (Sollentuna, Sweden) were used as wild-type controls for the IL-6^{-/-} mice. The animals were maintained under standardized environmental conditions, i.e. 24-26°C, 50-60% relative humidity, artificial lighting at 05.00-19.00 h, with water and pelleted food *ad libitum*. All procedures regarding the mice were conducted in accordance with protocols approved by the institutions (Göteborg and Lund) and the local ethical committees on animal care.

Measurements of body weight and food intake

In examples 1-4 the body weight of the IL-6^{-/-} mice and wild type control female mice were recorded regularly. The crown-rump length and the transversal abdominal diameter were measured in anesthetized animals by dual x-ray absorptiometry (DEXA) using the Norland pDEXA Sabre (Fort Atkinson, WI, USA). Body mass index was then calculated for each mouse as body weight/crown-rump length². Visceral and subcutaneous obesity was also evaluated by computerized tomography (CT) at a level 5 mm

cranially of the junction between the L6 and S1 vertebrae.

In example 5 body weight was measured using a weighing scale (A & D Instruments, EK-200G). Food consumption was measured daily by weighing the food left over 24 h after the previous fillup. Basal food intake was measured during pre-treatment with saline injections before onset of the leptin treatment. Body weight and food intake was measured for 3 days after the end of leptin treatment.

10

Leptin measurement

Plasma leptin was determined with a recently described radioimmunoassay (Ahrén, B. et al. (1997) Regulation of plasma leptin in mice: Influence of age, high-fat diet and fasting, Am. J. Physiol. 273, R113-R120; Linco Research, St Charles, Mo, USA). The method uses a polyclonal rabbit antibody raised against recombinant mouse leptin, ^{125}I -labeled tracer prepared with recombinant mouse leptin and mouse leptin as standard. Rabbit anti-rabbit IgG was used for separation of bound and free leptin.

20

In example 5 tail blood samples were collected from young (4 months) and old (12 months) wild-type and IL-6^{-/-} male mice.

25

Differences between IL-6^{-/-} and IL-6^{+/+} control mice were determined by Student's t-test. When more than two groups were compared, statistics were calculated by one-way ANOVA followed by Student-Newman-Keuls multiple range test.

30

Example 1

IL-6^{-/-} knock-out male mice were not heavier than their wild type littermates at 2-5 months of age. However, the body weight of 9 months old IL-6^{-/-} male mice was higher than that of the corresponding wild type animals, as evident from Fig. 1 A. The physical appearance of male mice at 9-10 months of age clearly showed that

35

the IL-6 $-/-$ mouse was considerably fatter than a wild type control of the same age, as shown in Fig. 1 B). Computerized tomography (CT) of the abdomen clearly indicated that both visceral (intraabdominal) and subcutaneous fat mass were markedly increased in the IL-6 $-/-$ mice compared to the wild type control, as evident from Fig. 1 C.

Example 2

In this example the effects of IL-6 knock-out on body weight was studied at different ages in female mice. The body weight did not differ between wild type and knock-out female mice between two and five months of age, but between seven and nine months of age the body weight was significantly higher in IL-6 $-/-$ than in wild type $+/+$ mice, as seen in Fig. 2 A. The body mass index of 9-10 months old IL-6 knock-out female mice was higher than that of the corresponding wild type females, which is illustrated in Fig. 2B. The transversal abdominal diameter, as measured by DEXA, was also larger in IL-6 knock-out female mice than in wild type controls at 9-10 months of age (Fig. 2C).

Example 3

Thereafter the daily food intake for three consecutive days was studied for 11 months old IL-6 $-/-$ female mice compared to in wild type IL-6 $+/+$ controls. From the results shown in Fig. 3 it is clearly evident that the food intake was increased in the IL-6 $-/-$ mice compared to the controls.

Example 4

Serum triglyceride and cholesterol levels of 11 months old female IL-6 $-/-$ mice were compared to wild type IL-6 $+/+$ controls. As can be seen in Fig. 4 A the serum triglyceride was considerably higher in the IL-6 $-/-$ mice. Also the circulating levels of leptin were mark-

edly higher, i.e., about three times, compared to those of wild type mice, as seen in Fig. 4 B.

Example 5

5 15-month-old IL-6 ^{-/-} and wild-type males received intraperitoneal (ip) injections of leptin at doses of 120 µg/day or 240 µg/day or vehicle twice daily (at 08:30 and 17:00) for 3 consecutive days. Human leptin was obtained from PeproTech (Rocky Hill, NJ, USA) and dissolved
10 in sterile PBS, 0.1% BSA. In order to get the animals used to injections, mice were given saline injections twice daily before the start of the leptin treatment.

The descriptive statistical results are presented as means ± SEM. Independent t test was used to test between-
15 group differences. Within-group differences were analysed using paired t test followed by the Bonferroni correction. *P* < 0.05 was considered significant.

Effects of leptin treatment on food intake

20 Vehicle treatment (PBS, 0.1% BSA) showed no effect on food intake compared to baseline levels in wild-type and IL-6 ^{-/-} mice (Fig. 6 A).

In contrast, treatment with leptin at a dose of 120 µg/day to wild-type male mice led to a 40% decrease
25 in food intake during the first two treatment days compared to baseline levels (baseline level: 4.91 ± 0.08 g). Food intake was not significantly decreased in IL-6 ^{-/-} mice during treatment with leptin in this dose (Fig 6 B). The decrease in food intake was significantly
30 larger in wild-type mice than in IL-6 ^{-/-} mice on day 1-3 of leptin treatment (Fig 6 B). At the end of the leptin treatment, food intake was normalised within 2 days in wild-type mice.

Leptin treatment at a larger dose (240 µg/day) led
35 to a reduction of food intake in wild-type males with the largest decrease (50%) from baseline level during the third treatment day (baseline level: 4.46 ± 0.30 g, Fig

8 C). There was no decrease in food intake in the IL-6 ^{-/-} mice (Fig 6 C). Three days after the end of the leptin treatment, food intake increased significantly to above baseline levels in wild-type mice and there was a similar
5 tendency in IL-6 ^{-/-} mice (Fig 6 C).

Effects of leptin treatment on body weight

Vehicle treatment (PBS, 0.1% BSA) showed no effect on body weight compared to baseline levels in wild-type
10 and IL-6 ^{-/-} mice (Fig 7 A).

However, body weights were markedly reduced during and after leptin treatment (120 µg/day) in wild-type mice, while the effect was less pronounced in the IL-6 ^{-/-} mice (Fig 7 B). The reduction in body weight was signifi-
15 cantly larger in wild-type mice than IL-6 ^{-/-} mice day 1-4 after initiation of leptin treatment.

Body weights were significantly reduced in wild-type mice both for three days during and for three days after a higher dose of leptin treatment (240 µg/day, Fig 9 C).
20 There was a tendency towards decreased body weights in leptin treated IL-6 ^{-/-} mice, but this decrease was not significant tested with paired t test followed by the Bonferroni correction for five comparisons. On day 3 of leptin treatment, the decrease in body weight was sig-
25 nificantly smaller in IL-6 ^{-/-} mice than in wild-type mice.

Discussion

In has thus been shown that IL-6^{-/-} mice have de-
30 creased responsiveness to leptin treatment compared to wild type mice. These findings indicate that presence of endogenous IL-6 is of importance for normal leptin responsiveness. Leptin treatment induced a significant reduction in food intake in the wild-type mice, but not in
35 the IL-6 ^{-/-} mice. In addition, the suppressive effect of leptin on body weight was less pronounced in IL-6 ^{-/-} mice than in wild-type mice. These effects of IL-6 may be re-

lated to the IL-6 receptor structure, since it has been shown that IL-6 type cytokine receptors share functional specificity with the long form of the leptin receptors (Ob-Rb, Baumann H et al., 1996). The receptor subunits for ciliary neurotrophic factor (CNTF) have been shown to share sequence similarities with Ob-Rb, Gloaguen I et al., 1997) and IL-6 receptors. When administered systemically, CNTF can reverse obesity in various animal models, including *db* mice lacking leptin receptors (Gloaguen I et al., 1997). All three of these systems, leptin, IL-6 and CNTF, signals through the JAK-STAT pathway to regulate gene expression (Flier JS, 1997; Hirano T, 1998; Gloaguen I et al., 1997). Cross-reactivity between the three systems at the receptor or post-receptor level may serve as an explanation for the link between regulation of body weight by leptin and IL-6 (as well as CNTF).

It has also been shown that the body weights of the IL-6 ^{-/-} mice in this study were significantly higher compared with the body weights of wild-type mice. This result is supported by the recent finding that IL-6 ^{-/-} mice develop "middle age onset" obesity (Wallenius V and Jansson JO, unpublished results). There may be several possible reasons why the obese phenotype of these mice has not been noticed previously. IL-6 ^{-/-} mice are commonly used to investigate the role of IL-6 in various infectious and inflammatory models (Kopf et al. 1994), but the weight gain in the IL-6 ^{-/-} mice was not observed until they were "middle aged", that is about 4 months of age. Younger animals are preferred for studying infection and inflammation. Moreover, the IL-6 ^{-/-} mice in this study were back-crossed for 8 generations to a 99.5% pure C57BL/6 background, which may be of importance for the development of the obese phenotype. If so, this raises the question whether the obese phenotype is exclusive for IL-6 ^{-/-} mice with a C57BL/6 background or if it also would be seen in other mice strains deficient for IL-6.

The weight gain in the IL-6 ^{-/-} mice could be secondary to the development of leptin resistance indicated by this study. If this is the case, one could expect the IL-6 ^{-/-} mice to have a higher level of basal food intake compared to wild-type mice. So far, studies on basal food intake in IL-6 ^{-/-} mice have not shown such results. There are also indications in the literature, suggesting that IL-6 affects energy expenditure rather than feeding (Chrousos GP, 1995). If IL-6 acts mainly on the regulation of energy expenditure relative to the regulation of appetite/food intake, the finding in this study that endogenous IL-6 may potentiate the suppressive effect of leptin on food intake is a bit surprising (Friedman JM and Halaas JL, 1998). It is common knowledge that food intake and appetite is reduced during infectious diseases and inflammation, conditions which are associated with increased levels of circulating IL-6 (Hirano T, 1998). However, there have been few earlier indications that the low basal production of IL-6 in healthy animals would affect food intake or fat mass. So far, the reason for the weight gain in the IL-6 ^{-/-} mice is not clear and needs further investigation.

Measurement of plasma leptin levels in male IL-6 ^{-/-} mice and wild-type male mice showed no significant difference between old IL-6 ^{-/-} mice and old wild-type mice. This is surprising for two reasons. Firstly, the IL-6 ^{-/-} mice were heavier than the wild-type mice because of increased body fat mass (Wallenius V and Jansson JO, unpublished results). Since plasma leptin levels are highly correlated with adipose tissue mass (Friedman JM and Halaas JL, 1998), the plasma leptin levels of the IL-6 ^{-/-} mice were expected to be higher than in the wild-type mice. Secondly, leptin resistance in the IL-6 ^{-/-} mice, as indicated by this study, is associated with increased plasma leptin levels. For instance, elevation of plasma leptin is seen in most obese humans with leptin resistance (Flier JS and Foster DW, Williams textbook of endo-

crinology 9th edition). Other measurements of plasma leptin levels in female mice have shown increased levels in the IL-6 ^{-/-} mice compared to wild-type mice (Wallenius V and Jansson JO, unpublished results). It is known that the levels of circulating leptin are higher in females than in males (Flier JS and Foster DW, Williams textbook of endocrinology 9th edition), and there are several gender differences in the regulation of fat mass (Vettor R et al., 1997). Therefore, the preliminary results of the measurements of plasma leptin levels in male IL-6 ^{-/-} mice need to be repeated and investigated further.

Example 6

In this example, the increase in body fat caused by IL-6 deficiency was confirmed by fat dissections in 18-month-old male (shown Fig. 8 A) and female (shown in Fig. 8 B) mice. Four different fat pads were dissected from these mice. The male and female IL-6 ^{-/-} and IL-6 ^{+/+} mice were first weighed and then three intra-abdominal fat pads (gonadal, retroperitoneal and mesenteric) and the femoral fat pad (subcutaneous pad in the groin of the thigh) were dissected and weighed. All investigated fat pads, except the male mesenteric fat pad (Fig. 8 A), were significantly larger in the IL-6 ^{-/-} mice compared to IL-6 ^{+/+} mice. In both males and females the total weight of all dissected fat pads was increased by 50-60 % in IL-6 ^{-/-} compared to IL-6 ^{+/+} mice (not shown).

Example 7

In this example female IL-6 ^{-/-} and IL-6 ^{+/+} mice were treated with IL-6 to see if it was possible to reverse some of the phenotypical changes observed in the IL-6 ^{-/-} mice. Figure 9 A shows that 18 days of IL-6 treatment reduced body weight to a larger extent in IL-6 ^{-/-} mice than in IL-6 ^{+/+} mice. Quantification of several CT scans performed before the start of IL-6 treatment showed that the intraperitoneal area was significantly higher in the IL-

6^{-/-} mice compared to the IL-6^{+/+} (Fig. 9 B). After 18 days of IL-6 treatment the total abdominal area had decreased significantly in the IL-6^{-/-} mice while there was no such effect in the IL-6^{+/+} mice (Fig. 9 B). Intraperitoneal areas were also measured, and they had a similar attenuation on the CT scans as subcutaneous fat. This quantification, excluding non-fat tissues, indicated an even larger increase in the fat content in IL-6^{-/-} mice compared to the IL-6^{+/+} mice (Fig. 9 C). There was a significant decrease in the intraperitoneal areas with fat-like attenuation after IL-6 treatment to the IL-6^{-/-} mice (Fig. 9 C). Before IL-6 treatment, leptin levels were almost three times higher in the IL-6^{-/-} mice compared to the IL-6^{+/+} mice. IL-6 replacement for 18 days to the IL-6^{-/-} mice caused a significant decrease in leptin levels compared to before treatment.

The computerized tomographies (CTs) in this example were performed with the Stratec peripheral quantitative computerized tomography (pQCT) XCT Research M (software version 5.4B; Norland Medical Systems Inc., Fort Atkinson, WI) operating at a resolution of 70 μ m. The section was made at the same point in all mice, i.e. 5 mm proximally of the crista illiaca.

CLAIMS

1. Use of a substance that upon administration to a patient will lead to an increased level of an interleukin-6 (IL-6) receptor agonist for the production of a medicinal product for treatment of obesity and/or obesity associated disorders.

2. Use according to claim 1, wherein said substance is an IL-6 receptor agonist.

3. Use according to claim 2, wherein said substance is IL-6.

4. Use according to any one of the claims 1-3, wherein said obesity and/or obesity associated disorders is caused by a pathological disturbance of fat metabolism.

5. Use according to claim 4, wherein said obesity is mainly visceral or intraabdominal.

6. Use according to any one of the claims 1-5, wherein said obesity is observed despite high levels of circulating leptin.

7. Use according to any one of the claims 1-6, wherein said obesity is accompanied by leptin insensitivity.

8. Use according to any one of the claims 1-3, wherein said disorder is a pathological increase of serum triglycerides.

9. Use according to any one of the claims 1-8, wherein said medicinal product is suitable for treatment of a cardiovascular disease.

10. Use according to any one of the claims 1-8, wherein said medicinal product is suitable for treatment of the metabolic syndrome.

11. Use according to any one of the claims 1-8 or 10, wherein said medicinal product is suitable for treatment of diabetes type II.

25. A method according to any one of the claims 17-24, wherein said medicinal product is suitable for treatment of a cardiovascular disease.

26. A method according to any one of the claims 17-
5 25, wherein said medicinal product is suitable for treat-
ment of a condition due to ageing.

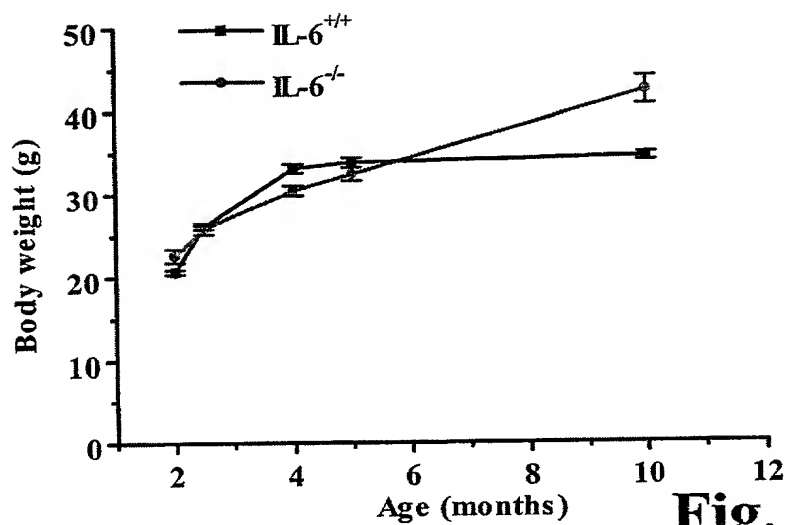
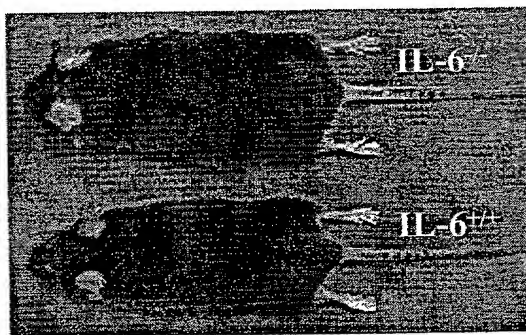
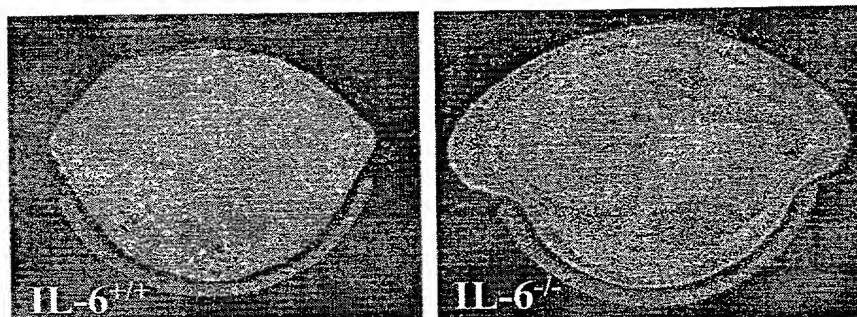
27. A method according to claim 26, wherein said patient is a human of the age 30 years or older.

28. A method according to any one of the claims 17-
10 27, wherein said IL-6 receptor agonist is administered in
combination with a factor that will intensify the effect
of said IL-6 receptor agonist.

29. A method according to claim 28, wherein said factor is a factor acting via gp130.

15 30. A method according to claim 28, wherein said
factor is leptin.

1/9

**Fig. 1 A****Fig. 1 B****Fig. 1 C**

2/9

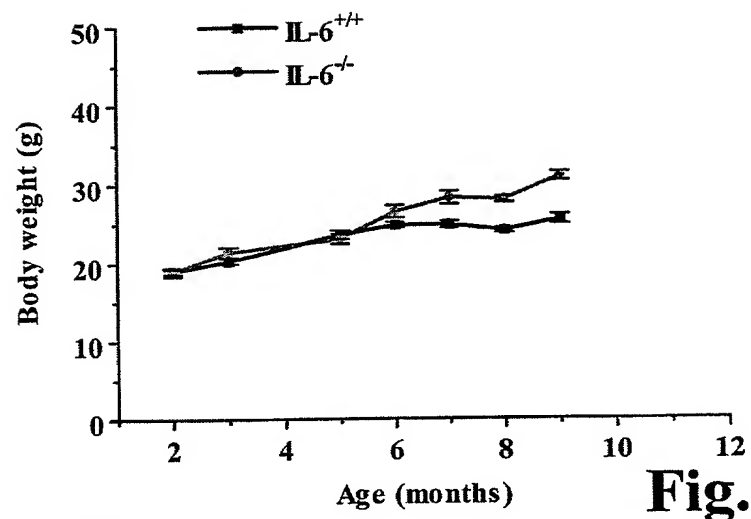


Fig. 2 A

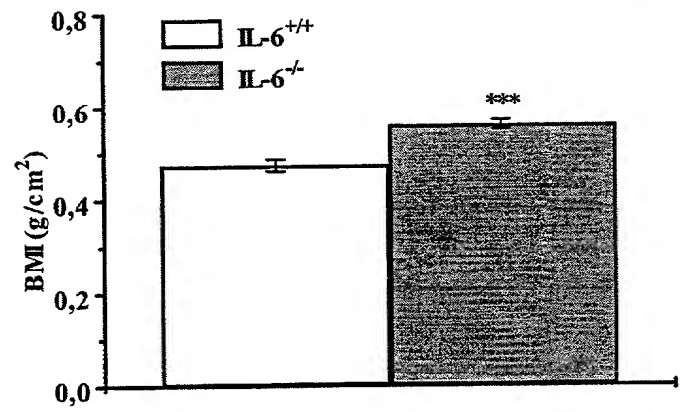


Fig. 2 B

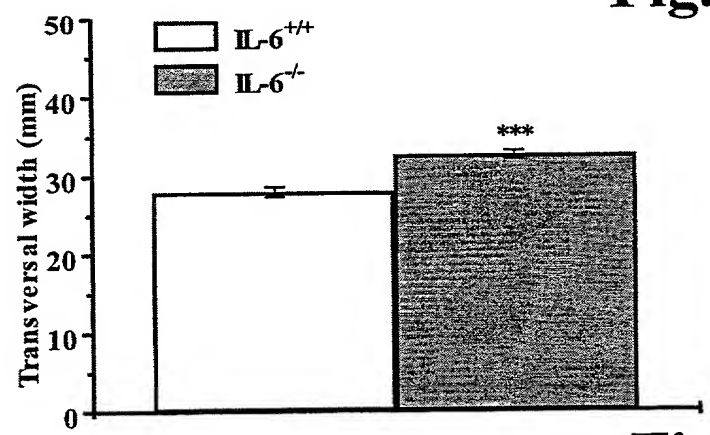


Fig. 2 C

3/9

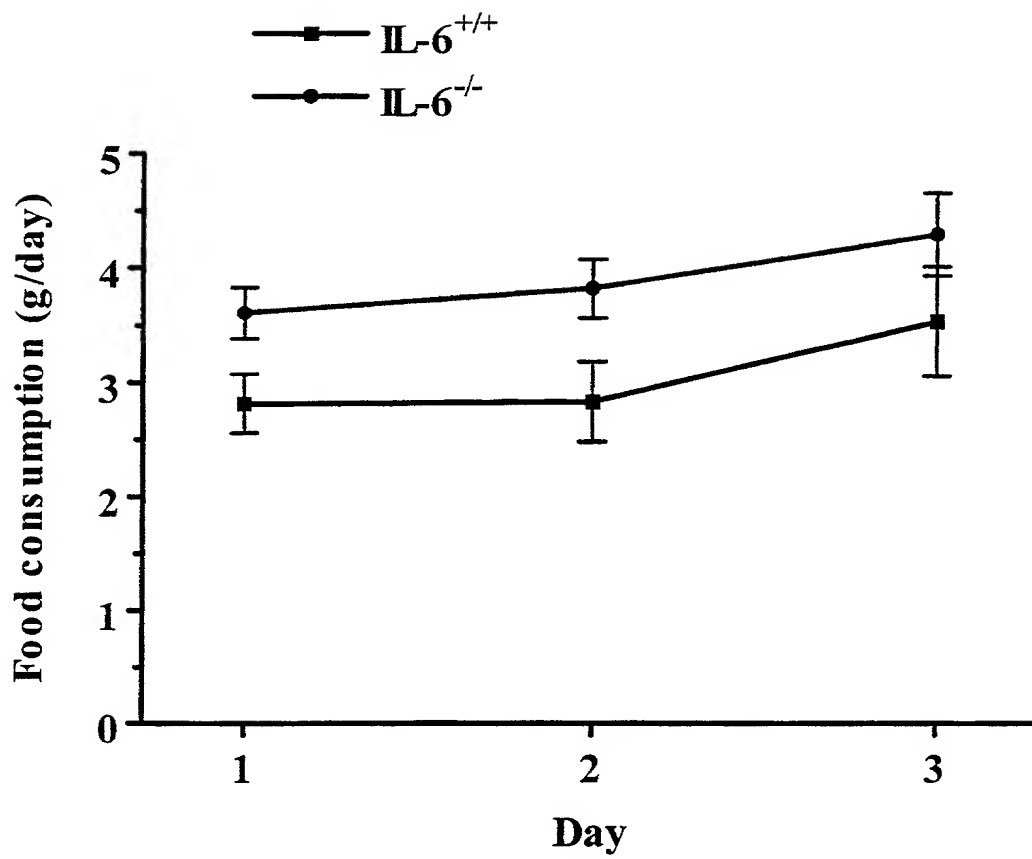
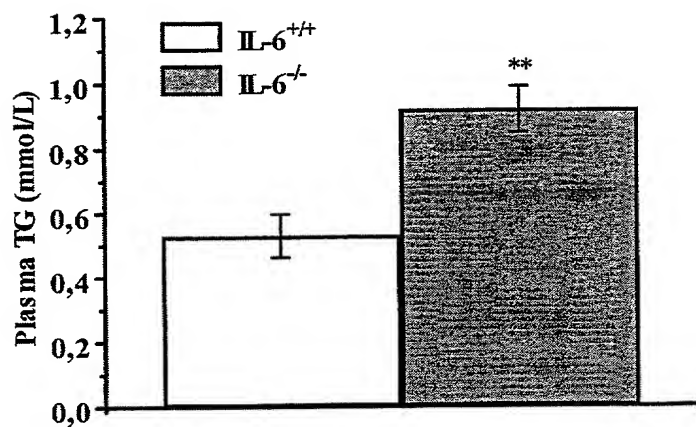
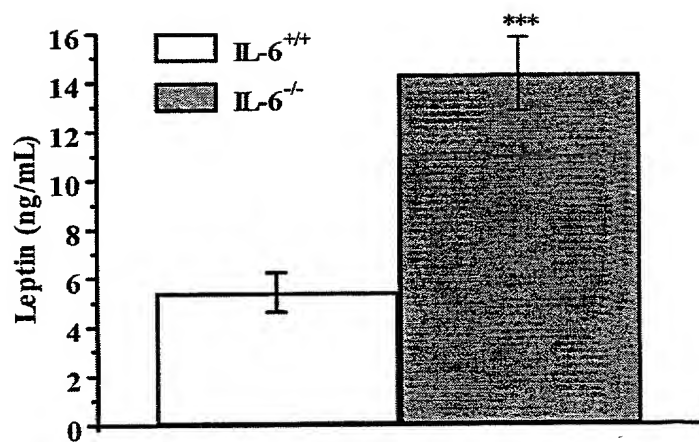
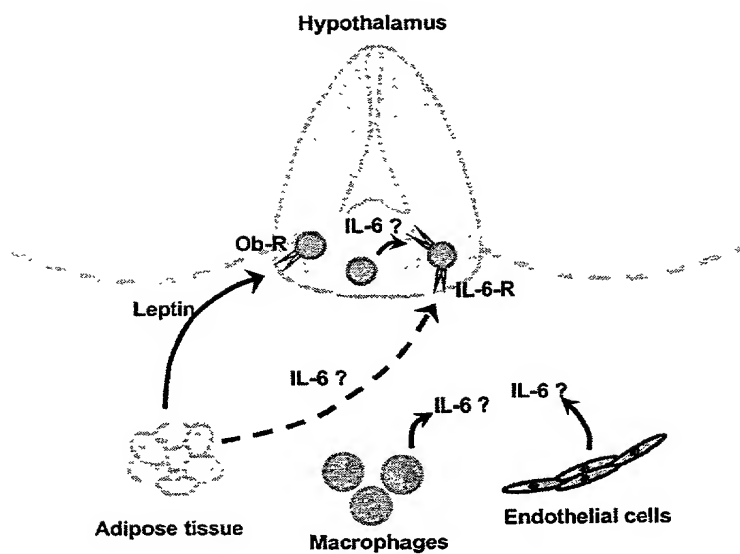


Fig. 3

4/9

**Fig. 4 A****Fig. 4 B**

5/9

**Fig. 5**

6/9

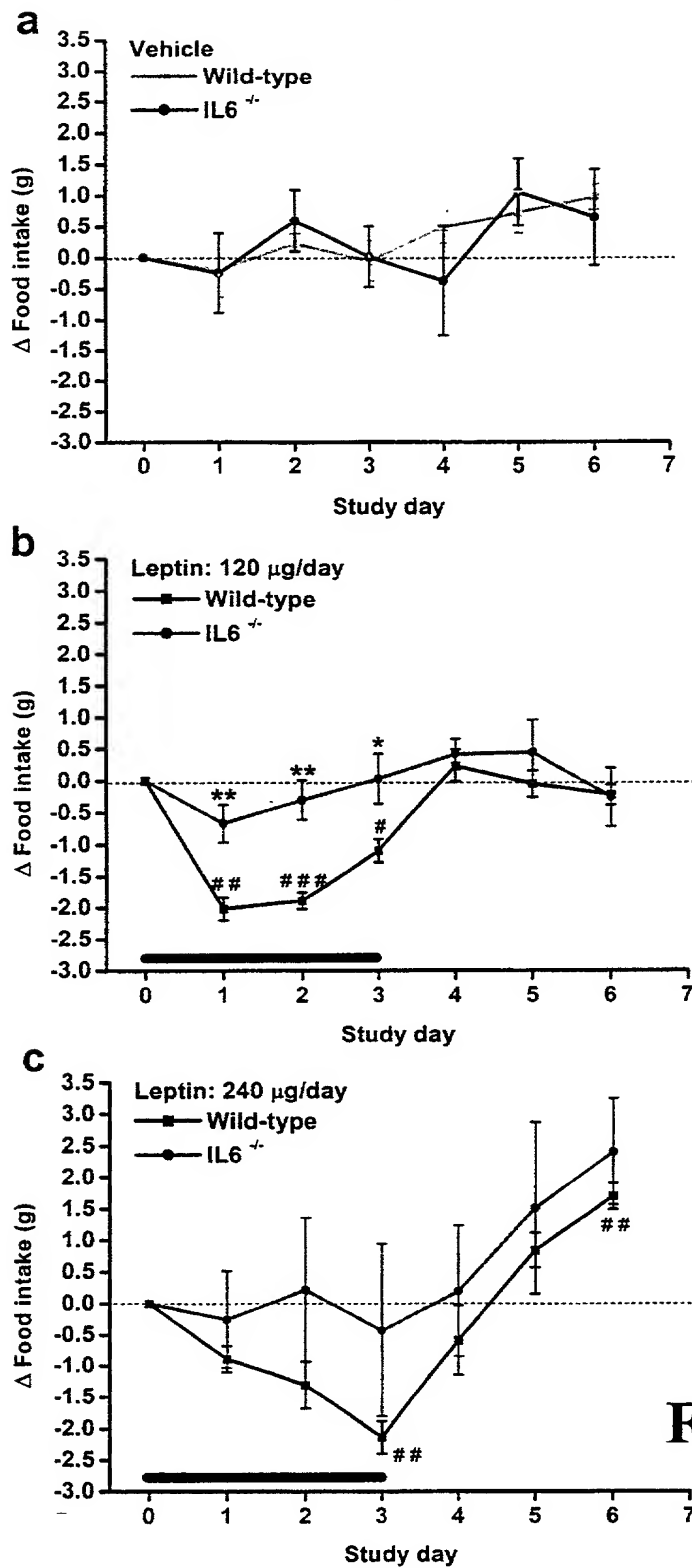
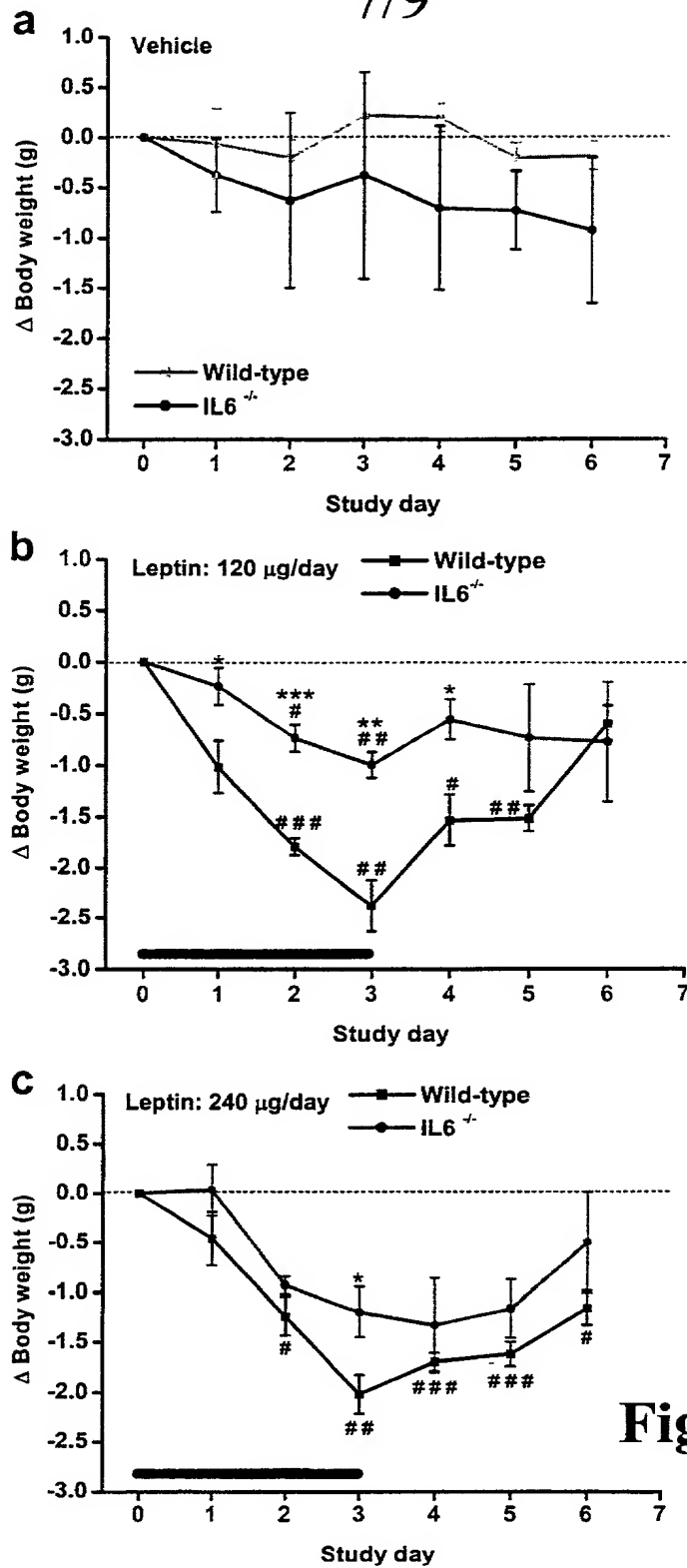


Fig. 6

7/9



8/9

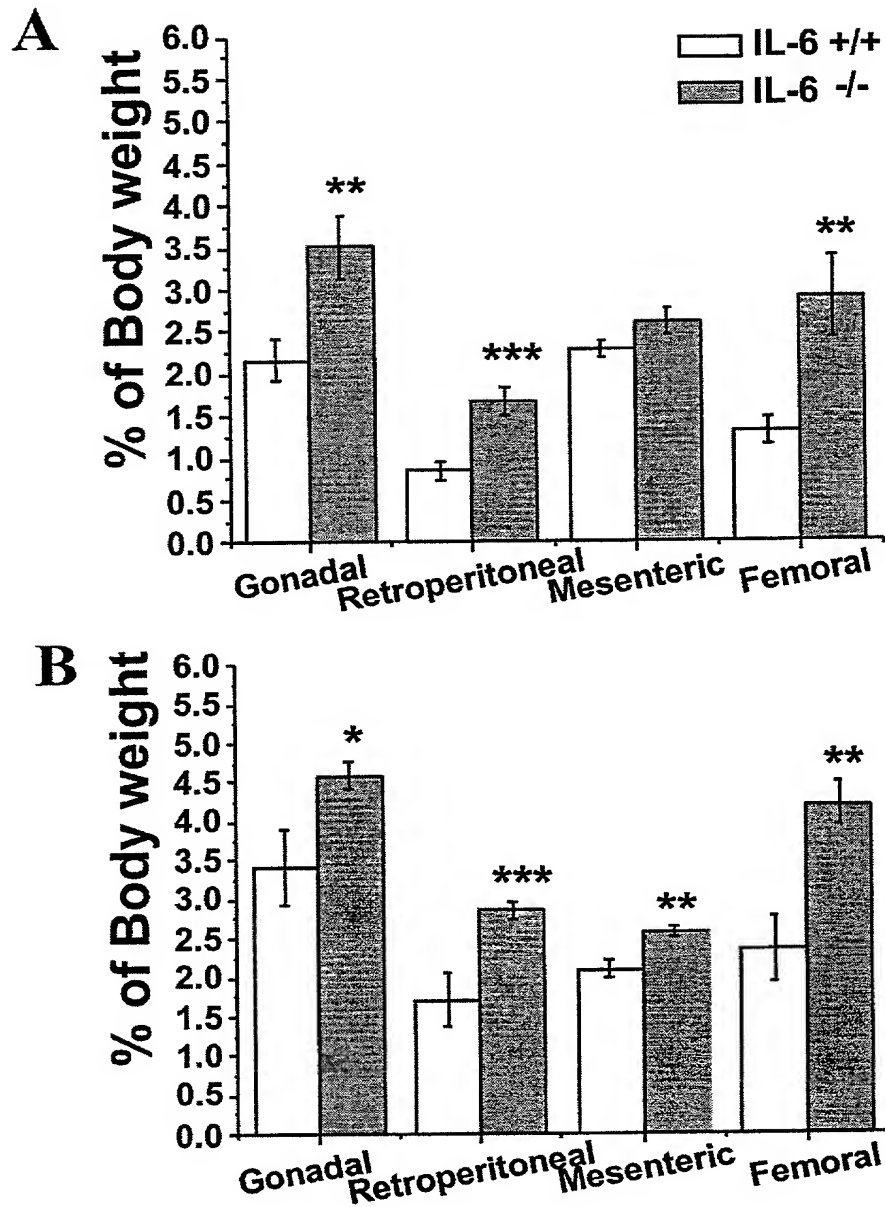


Fig. 8

9/9

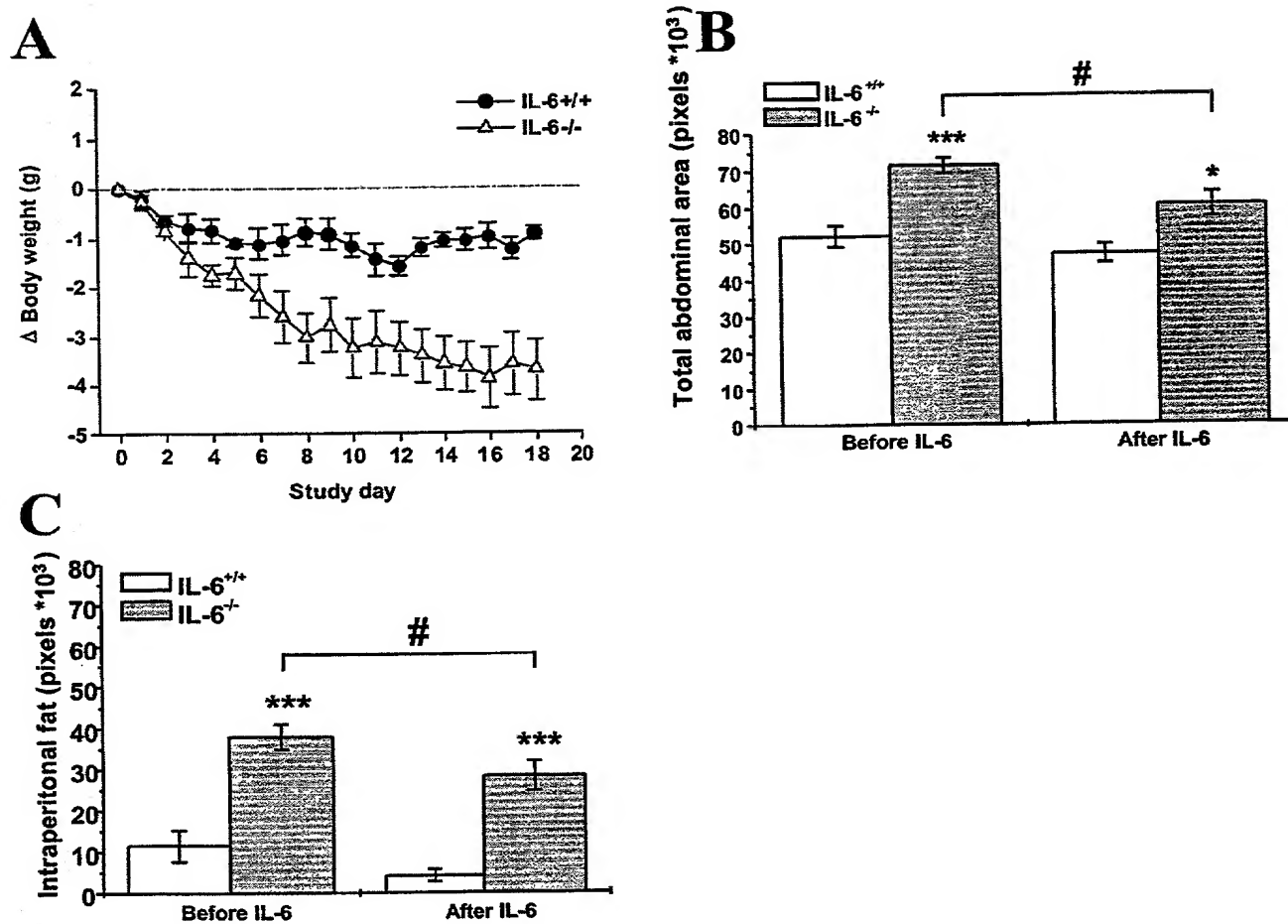


Fig. 9

**COMBINED DECLARATION AND POWER OF ATTORNEY
FOR UTILITY PATENT APPLICATION**

Attorney's Docket No.

003300-891

As a below-named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name;

I BELIEVE I AM THE ORIGINAL, FIRST AND SOLE INVENTOR (if only one name is listed below) OR AN ORIGINAL, FIRST AND JOINT INVENTOR (if more than one name is listed below) OF THE SUBJECT MATTER WHICH IS CLAIMED AND FOR WHICH A PATENT IS SOUGHT ON THE INVENTION ENTITLED:

USE OF INTERLEUKIN-6 IN TREATMENT OF OBESITY AND/OR OBESITY ASSOCIATED DISORDERS

the specification of which

(check one)

☐

is attached hereto;

☒

was filed on July 13, 2000

as

Application No. PCT/SE00/01491

and was amended on _____;

(if applicable)

I HAVE REVIEWED AND UNDERSTAND THE CONTENTS OF THE ABOVE-IDENTIFIED SPECIFICATION, INCLUDING THE CLAIMS, AS AMENDED BY ANY AMENDMENT REFERRED TO ABOVE;

I ACKNOWLEDGE THE DUTY TO DISCLOSE TO THE OFFICE ALL INFORMATION KNOWN TO ME TO BE MATERIAL TO PATENTABILITY AS DEFINED IN TITLE 37, CODE OF FEDERAL REGULATIONS, Sec. 1.56 (as amended effective March 16, 1992);

I do not know and do not believe the said invention was ever known or used in the United States of America before my or our invention thereof, or patented or described in any printed publication in any country before my or our invention thereof or more than one year prior to said application; that said invention was not in public use or on sale in the United States of America more than one year prior to said application; that said invention has not been patented or made the subject of an inventor's certificate issued before the date of said application in any country foreign to the United States of America on any application filed by me or my legal representatives or assigns more than twelve months prior to said application;

I hereby claim foreign priority benefits under Title 35, United States Code Sec. 119 and/or Sec. 365 of any foreign application(s) for patent or inventor's certificate as indicated below and have also identified below any foreign application for patent or inventor's certificate on this invention having a filing date before that of the application(s) on which priority is claimed:

COMBINED DECLARATION AND POWER OF ATTORNEY

Attorney's Docket No.

003300-891

COUNTRY/INTERNATIONAL	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED
Sweden	9902680-9	13 July 1999	YES <input checked="" type="checkbox"/> NO <input type="checkbox"/>
Sweden	9904424-0	03 December 1999	YES <input checked="" type="checkbox"/> NO <input type="checkbox"/>

I hereby appoint the following attorneys and agent(s) to prosecute said application and to transact all business in the Patent and Trademark Office connected therewith and to file, prosecute and to transact all business in connection with international applications directed to said invention:

William L. Mathis	17,337	Eric H. Weisblat	30,505	Bruce T. Wiedner	33,815
Robert S. Swecker	19,885	James W. Peterson	26,057	Todd R. Walters	34,040
Platon N. Mandros	22,124	Teresa Stanek Rea	30,427	Ronni S. Jillions	31,979
Benson S. Duffett, Jr.	22,030	Robert E. Krebs	25,885	Harold R. Brown III	36,341
Norman H. Stepno	22,716	William C. Rowland	30,888	Allen R. Baum	36,086
Ronald L. Grudziecki	24,970	T. Gene Dillahunty	25,423	Brian P. O'Shaughnessy	32,747
Frederick G. Michaud, Jr.	26,003	Patrick C. Keane	32,858	Kenneth B. Leffler	36,073
Alan E. Kopecki	25,813	B. Jefferson Boggs, Jr.	32,344	Fred W. Hathaway	32,236
Regis E. Stuner	26,999	William H. Benz	25,952	Wendi L. Weinstein	34,456
Samuel C. Miller, III	27,360	Peter K. Skiff	31,917	Mary Ann Dillahunty	34,576
Robert G. Mukai	28,531	Richard J. McGrath	29,195	Donna M. Meuth	36,607
George A. Hovanec, Jr.	28,223	Matthew L. Schneider	32,814	Mark R. Kresloff	42,766
James A. LaBarre	28,632	Michael G. Savage	32,596		
E. Joseph Gess	28,510	Gerald F. Swiss	30,113		
R. Danny Huntington	27,903	Charles F. Wieland III	33,096		



21839

and:

Address all correspondence to:

Benton S. Duffett, Jr.
BURNS, DOANE, SWECKER & MATHIS, L.L.P.
P.O. Box 1404
Alexandria, Virginia 22313-1404



21839

Address all telephone calls to: Benton S. Duffett, Jr. at (703) 836-6620.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

FULL NAME OF SOLE OR FIRST INVENTOR	SIGNATURE	DATE
JOHN-OLOV JANSSON	<i>[Signature]</i>	
RESIDENCE	CITIZENSHIP	
Donsö, Sweden	Sweden	
POST OFFICE ADDRESS		
Lebergsvägen 8, SE-430 82 Donsö, Sweden		
FULL NAME OF SECOND JOINT INVENTOR, IF ANY	SIGNATURE	DATE
VILLE WALLENLUS	<i>[Signature]</i>	31 01 02
RESIDENCE	CITIZENSHIP	
Göteborg, Sweden	Sweden	
POST OFFICE ADDRESS		
Sanatoriegatan 12B, SE-416 53 Göteborg, Sweden		